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(54) Clostridial vaccines containing tetramisole/levamisole as adjuvant

(57) The immune response of ruminant animals such as sheep and cattle to vaccination with Clostridial vaccines or a mixture of (i.e multivalent) Clostridial vaccines is improved using the imidazo [2,1-B]thiazole, tetramisole or levamisole and their pharmaceutically acceptable acid addition salts.

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SPECIFICATION

Improving the immune response of ruminants

This invention relates to a process for improving the immune response of ruminant animals, in particular sheep and cattle, to vaccination with Clostridial vaccines using the imidazo [2,1-*b*] thiazole tetramisole and its pharmaceutically acceptable acid addition salts.

In 1971 G Renoux and M Renoux reported (Comptes Rendus de l'Academie des Science, *272*, 349-355) improved protection against challenge with *Brucella abortus* in vaccinated mice which had been treated with tetramisole. Since that time there have been numerous publications relating to the use of tetramisole and its laevorotatory isomer levamisole, as a vaccine adjuvant. Some of the publications have reported that tetramisole or levamisole has shown an immunostimulating effect, others have reported that there is no effect and still others have reported an immunosuppressant effect. For example, the following references report that tetramisole or levamisole improve the response to vaccination:

(i) against *Brucella abortus* in mice, G REnoux and M Renoux, Infect. Immun., *8*, 544-548 (1973);
(ii) against *Plasmodium bergei* in rats, R S Desowitz, Exp. Parasitol., *38*, 6-13 (1975); and
(iii) against *Corynebacterium pseudotuberculosis* in mice, M R Irwin and H D Knight, Infect. Immun. *12*, 1098-1103 (1975).

However, the following references report that tetramisole or levamisole have no effect on the response to vaccination:

(iv) against pleuropneumonia, rinderpest and anthrax in cattle, A Provost, G Tacker and C Borredon, Rev. Elev. Med. Vet. Pays Trop., *27*, 39-52 (1974);
(v) against foot and mouth disease in cattle, C Rosenbusch and L M Schmied, Rev. Med. Vet., *54*, 467-471 (1973); and
(vi) against brucellosis in cattle and rabbits, A.I. De Diego et al, Gac. Vet., *36*, 164-170 and 584-588 (1974).

Moreover, the following references report that tetramisole or levamisole has a mild suppressant effect on the response to vaccination:

(vii) against *Corynebacterium pseudotuberculosis* in sheep, C M Cameron, Onderstepoort J. Vet. Res., *44*(1), 47-48 (1977); and
(viii) against infectious bovine rhinotracheitis in cattle, M R Irwin et al, Am. J. Vet. Res. *37*, 223-226 (1976).

This confusing mass of information shows that the effect of tetramisole or levamisole on an animal's response to vaccination is not predictable. Therefore, it is not obvious whether there is any advantage to be gained by utilizing tetramisole or levamisole as a vaccine adjuvant when immunizing particular species of animal against a particular disease, and no commercial vaccine has used tetramisole or levamisole as an adjuvant.

It has now been found that the response of ruminant animals to vaccination with Clostridial vaccines is significantly improved by the use of tetramisole as a vaccine aduvant and that this effect may be put to commercial use in protecting rumiants from infection by Clostridial diseases.

Therefore according to the present invention there is provided the use of an agent chosen from tetramisole, levamisole and the acid addition salts thereof as an adjuvant in conjunction with a Clostridial vaccine to improve the response of ruminant animals to said vaccine.

The invention also provides a process for improving the response of a ruminant animal to a Clostridial vaccine which comprises administering to said ruminant an effective amount of an agent chosen from tetramisole, levamisole and the acid addition salts thereof.

Suitable vaccines include the anaerobic vaccines which are used for the prevention of Clostridial diseases in sheep and cattle. Such vaccines include, for example, those which contain antigens prepared from strains of Clostridia such as *Clostridium welchii (Clostridium perfringens)* type B, C and D, *Clostridium septicum, Clostridium tetani, Clostridium chauvoei* and *Clostridium novyi (Clostridium oedematiens)* type B which are used in the treatment of Lamb dysentery, Pulpy Kidney disease (enterotoxaemia), Malignant Oedema (blood poisoning), Tetanus, Blackleg disease and Black disease, and combinations of two or more of these antigens.

Preferably the agent used to improve the response of ruminants to vaccination with a Clostridial vaccine is an acid addition salt of levamisole. Suitable levamisole salts include the hydrochloride, acetate, citrate, tartrate and phosphate salts. Preferably the hydrochloride or dihydrogen phosphate salt is used.

The agent used to improve the response of ruminants to vaccination with a Clostridial vaccine may be administered to the animal prior to, at the same time as, or subsequent to the administration of the vaccine. The agent may be administered to the animal by oral drenching but is preferably administered parenterally in the same manner as the vaccine is administered. When the agent is administered at substantially the same time as the vaccine it is preferable, and convenient, to administer the agent in admixture with the vaccine so that both the vaccine and the agent can be administered in the one operation.

The term "parenteral" is used herein to mean intravenous, intramuscular and subcutaneous injection. Preferably the compositions are administered according to the process of the invention by subcutaneous injection.

In aqueous solution tetramisole and levamisole readily undergo base catalysed hydrolysis. As a result aqueous formulations usually comprise a solution of an acid addition salt and are usually adjusted to an acid pH to provide the formulations with the required storage stability. For example, the aqueous formulations

disclosed in Australian Patents No 440,746 and 450,036 are adjusted to a pH of less than 4, and preferably 3.5.

It is well known that in order to maintain their activity vaccines should not be subjected to a pH of less than 6.0 or more than 7.0 and that as a general rule vaccines are unstable under conditions of low pH which promote protein denaturation. For example, J R Hepple "International Symposium on Adjuvants of Immunity, Utreckt 1966; Symp. Series Immunobiol. Standard", Vol 6 pp. 173-180, Karger, Basel/New York 1967, reports that with Clostridial vaccines it is important to maintain the pH in the range 6.1 to 6.4. The paper reports that too high a pH results in desorption of the antigen from the carrier while at low pH's denaturing of the antigens can occur, *Clostridium perfringens* type B and *Clostridium septicum* being sensitive to pH values below 6.0.

It has now been found, completely unexpectedly that aqueous compositions comprising Clostridial vaccines and tetramisole or levamisole are stable at acid pH, the efficacy of the vaccine component being unimpaired after long storage under the conditions normally employed to store such vaccines. As a result, when in the process of the invention the agent and the vaccine are administered in admixture, preferably the composition comprises a Clostridial vaccine component in admixture with an acid addition salt of tetramisole or levamisole at an acid pH. Preferably the pH is in the range of from 2.0 to 4.0, more preferably approximately 3.5.

Clostridial vaccines are normally prepared and stabilized in the presence of additives known as vaccine adjuvants. Thus the Clostridial vaccine compositions used in the process of the invention may, and preferably do comprise pharmaceutically acceptable adjuvants including preservatives and antigen carriers.

Suitable adjuvants include potassium alum, protamine, aluminium phosphate, aluminium hydroxide, calcium phosphate, glycerol, sorbitol, propylene glycol, carboxyvinyl polymers available under the Trade Mark "Carbopol" and bearing the designation 934, 940 and 941, Freund's universal adjuvant, soluble diethylaminoethyl (DEAE) dextran, saponin, "Quil-A", sodium chloride solution, and the fixed oils and synthetic esters of higher fatty acids which are known to be effective adjuvants.

Suitable preservatives include phenol, formaldehyde, propylene glycol, glycerol, esters of *p*-hydroxybenzoic acid, benzoic acid and its sodium salt, hexachlorophene, quaternary germicides and thiomersal as such or in the form in which it is available under the Trade Mark "Merthiolate".

Injectable anthelmintic compositions comprising tetramisole or levamisole in the form of their acid addition salts may comprise therapeutically acceptable salts, preferably at a concentration equivalent to from 0.1 to 0.15 moles per litre of solution, in order to prevent or reduce the incidence of tissue reaction at the site of injection. Thus when the agent used in the process of the invention to improve the response of ruminants to vaccination with a Clostridial vaccine is administered by subcutaneous injection the injectable composition may also comprise therapeutically acceptable salts. Suitable therapeutically acceptable salts include, for example, the sodium salts of citric, tartaric and phosphoric acid and mixtures thereof.

The dose rates employed in the process of the invention will vary with the ruminant animal being treated and the vaccine being used. However, in general the vaccine is administered at the same dose rate normally employed for the vaccination of the particular ruminant. The agent used to improve the response of a ruminant animal to vaccination with a Clostridial vaccine may be administered at a dose rate in the range of from 1 mg/kg of animal body weight up to the maximum non-toxic dose rate for the animal. Preferably, the agent is administered at the anthelmintic dose rate recommended for the animal. For example, in sheep, when levamisole is used as the agent it is preferably administered at a dose rate of approximately 10-17 mg/kg of animal body weight.

The process of the invention provides a surpisingly high improvement in the responses of ruminants to vaccination with Clostridial vaccines. For example, sheep treated according to the process of the invention with a composition comprising a multivalent Clostridial vaccine and the anthelmintic dose of levamisole dihydrogen phosphate showed the following statistically significant improvements in antitoxin titres when compared to controls treated with the vaccine alone:

Clostridial Antigen	% of Sheep with Antitoxin Titre above 5 units/ml	
	Control Sheep	Sheep Treated According to the Process of the Invention
Cl. welchii D (PK)	35	88
Cl. septicum (MD)	27	55
Cl. oedematiens (BD)	83	100
Cl. tetani (TET)	70	97
Cl. welchii C (LD)	60	97

This significant improvement in the response of ruminants to vaccination with Clostridial vaccines which is afforded by the process of the invention is completely unexpected, for other, non-ruminant, animals show

either no improvement in response or a lower response. For example, rabbits treated with a composition comprising a multivalent Clostridial vaccine and the anthelmintic dose of levamisole dihydrogen phosphate showed (see Table below) either no improvement in antitoxin titre or a reduced antitoxin titre when compared to controls treated with the vaccine alone.

Clostridial Antigen	Rabbit Antitoxin Titre (units/ml)	
	Vaccine only	Vaccine + Agent
Cl. welchii D	6.6-8.0	5-6.6
Cl. septicum	6.6-8.0	5-6.6
Cl. oedematiens	4-5	4-5
Cl. tetani	8-10	6.6
Cl. welchii C	10-13.3	6.6-8.0

It will be evident to those skilled in the art that the significant improvement in response of ruminants to vaccination with Clostridial vaccines which is afforded by the process of the invention will be of significant economic benefit in animal husbandry. Moreover, when in the process of the invention the agent is administered to the ruminant at the anthelmintic dose rate for that animal, and in admixture with the Clostridial vaccine, the advantage of the significant improvement in response of the ruminant to vaccination is combined with the advantage of protecting the ruminant from Clostridial disease and helminthiasis in the one operation with a saving in both time and labour.

This advantage may be put to particular benefit in the vaccination of pregnant ewes before lambing. Prior to the present invention it has been conventional procedure to treat pregnant ewes with an anthelmintic 4 to 6 weeks before lambing and then to vaccinate the ewes with a Clostridial vaccine 2 weeks before lambing. The vaccination close to lambing (ie 2 weeks before) has been necessary to ensure a "carry-over" of antibodies to the lamb which could not be achieved with earlier vaccination (ie 4 to 6 weeks before).

The significant improvement of response of ruminant animals to vaccination with a Clostridial vaccine which is afforded by the process of the present invention means that the vaccination of pregnant ewes can now be carried out at least 6 weeks before lambing with a "carry-over" of antibodies to the lamb similar to that achieved by the conventional prior art process of vaccination 2 weeks before lambing. As a result, the anthelmintic treatment and vaccination of pregnant ewes can be combined in the one operation 4 to 6 weeks before lambing with considerable cost saving to the farmer.

When a (L-) tetramisole salt and a Clostridial vaccine are formulated into a composition for use in the process of the present invention no loss in activity has been observed in the (L-) tetramisole component but a significant improvement has been observed in the efficacy of the vaccine component. Therefore, compositions are preferably formulated to contain, in a suitable dosage volume, the dose of (L-) tetramisole and the dose of vaccine usually employed in the treatment of that particular animal when the (L-) tetramisole and the vaccine are parenterally administered separately, as single therapeutic agents.

Such dose rates vary with the animal being treated and the specific (L-) tetramisole salt and vaccine being used. However, in general levamisole is administered at a dose rate of approximately 5 to 10 mg (calculated as the free base) per kilogram of animal bodyweight, D,L-tetramisole is administered at a dose rate of approximately 10 to 17 mg (calculated as the free base) per kilogram of animal body weight and in general vaccine preparations have been standardized to a dose volume of 2 ml for sheep and 4 ml for cattle for mono-, di-, tri-, tetra- and multivalent vaccines.

In combatting diseases by vaccination it is usual to administer two doses of vaccine to previously un-vaccinated animals the second dose being administered at least four weeks after the first dose. Thus in order to optimize the protection afforded by vaccination according to the process of the invention it is preferable to repeat the parenteral administration of a therapeutically effective amount of the vaccine at least four weeks later.

The compositions used in the process of the invention may comprise, in addition to the components hereinbefore defined: other pharmaceutically therapeutic agents such as, for example, flukicides, selenium (to combat white muscle disease) and systematically active pesticides; additives to improve the shelf life of the composition; buffering agents; preservatives; and/or additives to prevent or to reduce adverse tissue reaction at the site of the injection.

The invention is now illustrated, but not limited, by the following Examples.

Example 1

In order to evaluate the efficacy of the process of the invention an injectable composition was prepared by admixture of a seven component Clostridial vaccine comprising antigens prepared from *Clostridium welchii* Type B, *Clostridium welchii* Type C, *Clostridium welchii* Type D, *Clostridium septicum*, *Clostridium tetani*, *Clostridium chauvoei* and *Clostridium novyi* Type B [available from ICI Tasman Limited under the name "Tasvax" 7 ("Tasvax" is a Trade Mark] and an aqueous sterile filtered solution of levamisole dihydrogen

phosphate (17.6% w/w as free base). After admixture the pH of the composition was adjusted to 3.55. The make up of the test composition and the control composition comprising the 7 component Clostridial vaccine alone, are detailed in Table 1 below:

TABLE 1

Composition+ No.	Vaccine Component Volume (ml); pH	Tetramisole Component Volume (ml); pH	pH of Composition
A_1	500; 3.75	425; 3.5	3.55
Control 1	500; 6.3	- -	-

+ After information each composition was stored at 4° to 6°C in a glass bottle.

Example 2

This Example illustrates the efficacy of the process of the invention.

After storage for 6 months at a temperature of 4°C test composition A_1 and control composition C_1, prepared as detailed in Example 1, were tested for efficacy by injection into sheep following the dosing schedule detailed in Table 2 below. Each composition was tested on approximately forty sheep so that a meaningful statistical analysis of the results could be made. A control group of 6 sheep were not treated wih either the test composition A_1 or the control composition C_1.

TABLE 2

Day	Operation
1	a) Blood serum sample taken b) Group A_1 sheep injected with 3.5 ml of Composition A_1 Group C_1 sheep injected with 2.0 ml of Composition C_1
42	a) Blood serum sample taken b) Injections given on Day 1 were repeated
56	Blood serum sample taken

The antigenicity of the vaccines was evaluated by assaying the blood serum samples taken at 56 days for *Clostridium perfringens* Type D *Clostridium welchii* Type D; common name - pulpy kidney), *Clostridium septicum* (common name - malignant oedemia), *Clostridium novyi* Type B *(Clostridium oedematiens* Type B; common name - Black disease), *Clostridium tetani* (common name - tetanus) and *Clostridium perfringens* Type C (*Clostridium welchii* Type C; common name - lamb dysentery). The antitoxin titres were determined by conventional assay methods using mice.

The antitoxin titres on the pooled samples of sera from the sheep in each group are recorded in Table 3.

TABLE 3

Test Compositions	Antitoxin Titre+ (units/ml) PK	MO	BD	TET	LD
A_1	16-20	13.3-16	27-32	20-26.7	16-32
C_1	5-6.6	4	20-27	13.3	8.10
Untreated	<0.67	<0.67	<0.67	<0.67	<0.67

+ PK - pulpy kidney
MO - malignant oedemia
BD - Black disease
TET - tetanus
LD - lamb dysentery

For the purpose of statistical analysis antitoxin titres were determined on serum samples taken from the individual sheep. The results for the sheep treated with the test composition A_1 are given in Table 4 and the results for the sheep treated with the control composition C_1 are given in Table 5.

TABLE 4

Antitoxin Level of Sheep Treated with Test Composition (A_1)

Sheep Number	Antitoxin Titre (units/ml) PK	MD	BD	TET	LD
1	6.6	4	40-53	26-32	13-16
2	40-53	5-6.6	16-20	26-32	20
4	13.3	5-6.6	26-32	60-80	20-26.7
5	53-64	40	64-80	96-120	106
6	13-16	2-3.2	26-32	13-16	26.7
7	10-13	2	20	10-13	16-20
9	13-16	3.5	26	16-20	26.7
10	20-23	<1.0	8-10	5.3-6.6	5-8
11	4-5	5.3	8	3.2-5.3	5
12	2	2-3.2	20-26	20-26	8
13	6.6	5-6.6	32-40	20-26	20-26.7
14	3.3-4	2-3.2	16	20	13
15	32-40	32	64-80	96-120	80
16	6.6-8	2-3.2	32-40	32-40	8
17	16-20	20-27	26-32	53-64	26.7
18	40-53	8-10	64-80	64	20-26
19	5-6.6	3-5	10-13.3	13-16	3-5
20	32-40	8	32-40	32-40	20
21	26-32	80	26-32	40-53	10-13
22	3.3	5.3	13-16	16	10-13
24	13-16	27	32-40	32-40	13
26	10	27-32	20	26-32	20-26.7
27	10-13	10-13	53.4-64	60-80	20-26.7
28A	23-26	2-3.2	32-40	53-64	32-40
28B	8-10	5	20-26	20-26	8-10
29	3.3	1	13-16	10-13	5-10
30	13-16	1-2	40-53	32	16-20
31	40-53	40-48	10-13	32	32-40
32	13.3	16	13-16	32	8
33	13-16	32	20	32	6-8
34	16-20	26-32	32-40	40-48	40-53
35	16	3-5	64-80	48-60	64-80
36	10-13	3.3	20-26	26.7	10-13
39	13-16	3-5	64	48-60	32-40
46	40-53	2-3.2	20-26	20	13-16
41	13-16	6-8	32-40	26.7	10
49	10-13	6.6	32-40	60-80	53-64

TABLE 5

Antitoxin Level of Sheep Treated with Control Composition (C_1)

Sheep Number	Antitoxin Titre (unit/ml) PK	MD	BD	TET	LD
50	3.2-4	20-26.7	20-26.7	32-40	16-20
51	4.8-6	13.3	80-100	40-53	40-53
52	2-2.7	<2	6-8	3.2	No serum
53	3.2	1-2	3-5	2-3.2	No serum
54	1-2	2-4	64	20	16
55	3.2	2-4	10	10	16-20
56	1	1-2	20-26.7	3.3-5	3-5
57	2.7-3.2	1	16-20	5-8	3-5
58	2.7-3.2	2	6-8	8-10	10-13
59	10.13.5	6-8	10-13.3	10	2-3
60	16-20	2-4	13-16	3.3-5	8-10
61	3.4-4.7	1-2	5-8	2-3.2	<3
62	<0.67	<4	<3	<0.67	No serum
63	20-27	0.67-1.0	53	10	53
64	2	1-2	10-13	6.6-8	<0.67
65	2.7	5	20-26.7	10-13.5	20
66	16	2-4	40-53	27	27
67	6	2-4	40-53	10-13.5	6.6
68	4.8	2	20-26.7	40-53	6-8
69	16-20	4	20-26.7	20-27	6.8
70	1-2	<0.67	3.3-5	3-5	No serum
71	5-6.6	5-6.6	8.10	10-13.5	6.6
72	1-2	<0.67	10	3-5	No serum
73	5-6.6	1-2	8-10	3.3-5	20-26.7
74	0.67	<0.67	No serum	<2	No serum
75	<2	0.67-1	No serum	2-3.3	No serum
76	5-6.6	1-2	26-32	13.3	8
77	2-2.7	0.67-1.0	3-5	5-8	3-5
78A	6.6-8	8	10-13.3	16	No serum
78B	2.7	8	No serum	10-13.5	No serum
79	2-2.7	1.0	13.3	5-8	5
80	13.5-16	8-10	26-32	27-32	27
82	10-13.5	3-5	20-26	10-13.5	32
83	2	0.67-1.0	5-8	3.3-5	3-5
84	2-3.2	10.0	5-8	3.3-5	3-5
85	2	3-5	13.3	2-3.3	13.3
86	3.3-5	0.67	10-13.3	3.3-5	6-8
87	27-32	13.3	13.16	13.5-16	20
89	1-2	0.67-1.0	20	13.5	8-10

From the results presented in Tables 3, 4 and 5 it is evident that there was a marked improvement in the response of the sheep treated according to the process of the invention compared to the sheep treated with a Clostridial vaccine alone. The results were analysed by graphing each group of titre results for both the test composition sheep and the control composition sheep as a probability plot. In each case the improvement was shown to be statistically significant ($p < 0.001$).

In order to better illustrate the significance of the improvement given by the process of the invention the probability plots were used to determine (a) the percentage of sheep in each group having antitoxin levels of at least 5, 10, 20 and 40 units/ml and (b) the minimum antitoxin level in the sera of 75%, 50% and 25% of the sheep. The results are presented in Tables 6 and 7 respectively and clearly show the significant improvement in response to vaccination with Clostridial vaccines afforded by the process of the invention.

TABLE 6

Percentage of Sheep with a Given Antitoxin Level

Clostridial Antigen	Antitoxin Level (units/ml)	Percentage Sheep with Given Antitoxin Level	
		Test Composition (A_1)	Control Composition (C_1)
PK	5	88	35
	10	65	17
	20	35	6
	40	12	0
MD	5	55	27
	10	35	11
	20	20	2
	40	8	-
BD	5	100	83
	10	95	62
	20	75	30
	40	25	10
TET	5	97	70
	10	93	45
	20	75	20
	40	35	7
LD	5	97	60
	10	75	40
	20	40	20
	40	17	6

TABLE 7

Minimum Antitoxin Level in a Given Percentage of Sheep

Clostridial Antigen	Given % of sheep	Antitoxin Titre (units/ml)	
		Test Composition (A_1)	Control Composition (C_1)
PK	75	8	2
	50	14	4
	25	26	6
MD	75	3	1
	50	6	2
	25	16	6
BD	75	20	7
	50	28	13
	25	43	23
TET	75	20	4
	50	31	8
	25	46	16
LD	75	10	2
	50	17	7
	25	30	17

Example 3

This Example demonstrates the efficacy of the process of the invention in vaccinating pregnant ewes 6 weeks before lambing rather than the conventional 2 weeks before lambing.

For this experiment two different farms were used and ewes with a previous vaccination history were selected. At each site the animals were divided and tagged into two groups of 40 animals for test purposes and one group of 20 animals to serve as an untreated control group. After tagging the sheep were allowed to graze together as a mob.

A sterile injectable composition was prepared by combining 500 parts (0.53 standard dose units per part) of a pentavalent Clostridial vaccine comprising antigens from *Clostridium perfringens* Type D (Clostridium welchii Type D; common name - pulpy kidney), *Clostridium tetani* (common name - tetanus), *Clostridium chauvoei* (common name - blackleg), *Clostridium septicum* (common name - malignant oedemia) and *Clostridium novyi* Type B (*Clostridium oedematiens* Type B; common name - black disease) with an aqueous solution of levamisole dihydrogen phosphate (350 parts containing 18.2% w/v levamisole calculated as the free base) and adjusting the pH of the resulting composition to 3.5 by the addition of phosphoric acid. The resulting composition contained 0.31 standard dose units of vaccine per ml and 75 mg/ml of levamisole (calculated as the free base).

Groups IX and IIX sheep (site I and II respectively) were the control groups and remained untreated throughout the trial. The sheep in groups IY and IIY were each treated 6 weeks before lambing by subcutaneous injection with 3.5 ml of the combined Clostridial vaccine - levamisole dihydrogen phosphate composition prepared as described above. The sheep in groups IZ and IIZ were each treated 2 weeks before lambing by subcutaneous injection with 2.0 ml of a pentavalent Clostridial vaccine (portion of the same vaccine used in the preparation of the combined composition prepared as described above).

The dosing and bleeding schedule for the sheep is given in the Table below.

Weeks before (-) and after (+) lambing	Operation
−6	Serum sample taken
−6	Group IY and IIY sheep injected
−2	Serum sample taken
−2	Group IZ and IIZ sheep injected
+3	Serum sample taken from ewes and lambs
+7	Serum sample taken from ewes and lambs
+11	Serum sample taken from ewes and lambs

The sera samples from each sheep in a group were combined, as were the sera samples from each lamb in a group, and the antitoxin titres for pulpy kidney (PK), black disease (BD), malignant oedemia (MO) and tetanus (TET) were determined on the pooled sera samples by conventional assay methods using mice. The results are given in Table 8 below.

TABLE 8a

Antitoxin Titre on Pooled Sheep Sera

Group	Week of Bleed	Antitoxin Titre (units/ml) PK	BD	MO	TET
IX	-6	2	<0.67	<0.67	1-2
IY	-6	2	<0.67	<0.67	1-2
IZ	-6	2	<0.67	<0.67	1-2
IY	-2	16-20	4-5.3	1-2	8-10
IZ	-2	1-2	<0.67	<0.67	1-2
IY	+3	8-10	1-2	1.0	2-3.2
IZ	+3	4-5.3	2-3.2	0.8-1	2-3.2
IY	+7	3.2-4	0.8-1	0.6-1	2-3.2
IZ	+7	N/B	N/B	N/B	N/B
IY	+11	3.2	0.5-0.6	0.1-0.2	1-2
IZ	+11	1-2	0.5-0.6	<0.1	1-2
IIX	-6	<0.67	<0.67	<0.67	<0.67
IIY	-6	<0.67	<0.67	<0.67	<0.67
IIZ	-6	<0.67	<0.67	<0.67	<0.67
IIY	-2	13-16	2-3.2	1-2	2-3.2
IIZ	-2	<0.67	<0.67	<0.67	<0.67
IIY	+3	2.0	<0.35	<0.35	<0.35
IIZ	+3	1-2	0.67-1	0.5	0.3-0.5
IIY	+7	3.2	0.2-0.32	0.1-0.2	<0.1
IIZ	+7	N/B	N/B	N/B	N/B
IIY	+11	0.8-1.0	0.2	0.2-0.4	<0.13
IIZ	+11	N/B	0.2	0.2-0.4	0.1-0.2

N/B - Not Bled

TABLE 8b

Antitoxin Titre on Pooled Lamb Sera

Group	Week of Bleed	Antitoxin Titre (units/ml) PK	BD	MO	TET
IY	+3	5-6.6	1-2	1-2	3.2-4
IZ	+3	4-5.3	2-3.2	0.5-0.6	2-3.2
IY	+7	1-2	0.64-1	0.4	1-2
IZ	+7	1-2	0.4-0.64	0.32	0.64-1
IY	+11	0.8	0.2	<0.1	0.4-0.64
IZ	+11	0.5	0.2-0.4	<0.1	0.2-0.3
IIY	+3	5-6.6	<0.35	0.5	<0.35
IIZ	+3	3-5	1-2	0.6-0.8	0.67-1
IIY	+7	3.2	0.1-0.2	0.2	<0.1
IIZ	+7	1-2	0.64-1	0.4-0.64	0.32-0.4
IIY	+11	0.8-1.0	<0.06	0.1-0.2	<0.13
IIZ	+11	N/B	0.1-0.2	0.1-0.2	<0.1

N/B - Not Bled

CLAIMS

1. The use of an agent chosen from tetramisole, levamisole and the addition salts thereof as an adjuvant in conjunction with a Clostridial vaccine to improve the response of ruminant animal to said vaccine.

2. The use of an agent according to claim 1 wherein said Clostridial vaccine comprises antigens prepared from Clostridia chosen from the group *Clostridium welchii, Clostridium chauvoei, Clostridium septicum, Clostridium tetani, Clostridium chauvoei* and *Clostridium novyi* or a combination of one or more of said antigens.

3. The use of an agent according to claim 1 or claim 2 wherein said agent is an acid addition salt of levamisole.

4. The use of agent according to claim 3 wherein said acid addition salt is chosen from hydrochloride, acetate, citrate, tartrate or phosphate salts of levamisole.

5. The use of an agent according to claim 3 or claim 4 wherein said salt is the hydrochloride salt of levamisole.

6. The use of an agent according to claim 3 or claim 4 wherein said salt is the dihydrogen phosphate salt of levamisole.

7. A process for improving the response of a ruminant animal to vaccination with a Clostridial vaccine which process comprises administering to said ruminant an effective amount of an agent chosen from tetramisole, levamisole and the acid addition salts thereof.

8. A process according to claim 7 wherein said Clostridial vaccine comprises antigens prepared from Clostridia chosen from the group *Clostridium welchii, Clostridium chauvoei, Clostridium septicum, Clostridium tetani* and *Clostridium novyi* or a combination of one or more of said antigens.

9. A process according to claim 7 or claim 8 wherein said agent is an acid addition salt of levamisole.

10. A process according to claim 9 wherein said acid addition salt is chosen from hydrochloride, acetate, citrate, tartrate or phosphate salts of levamisole.

11. A process according to claim 9 or claim 10 wherein said salt is the hydrochloride salt of levamisole.

12. A process according to claim 9 or claim 10 wherein said salt is the dihydrogen phosphate salt of levamisole.

13. A process according to any one of claims 7 to 12 inclusive wherein said agent is administered to the ruminant prior to, at the same time as, or subsequent to the administration of the vaccine.

14. A process according to any one of claims 7 to 13 inclusive wherein said agent is administered in admixture with said Clostridial vaccine.

15. A process according to any one of claims 7 to 14 inclusive wherein said agent is administered parenterally.

16. A process according to any one of claims 7 to 15 inclusive wherein said agent is administered by subcutaneous injection.

17. A process according to any one of claims 7 to 16 inclusive wherein said agent is administered at a dose rate in the range of from 1 mg/kg of animal body weight to the maximum non-toxic dose rate for the animal.

18. A process according to any one of claims 7 to 17 inclusive wherein said agent is administered at the anthelmintic dose rate recommended for the animal.

19. A process according to any one of claims 7 to 18 inclusive wherein said ruminant animals are cattle.

20. A process according to any one of claims 7 to 19 inclusive wherein said ruminant animals are sheep.

21. A process according to any one of claims 7 to 18 inclusive wherein said agent is administered to pregnant ewes 4 to 6 weeks before lambing.

22. A process according to claim 7 substantially as herein described with reference to Examples 2 or 3.

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